Chronic Reduction of Nitric Oxide Level in Adult Spontaneously Hypertensive Rats Induces Aortic Stiffness Similar to Old Spontaneously Hypertensive Rats

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Key Words
Arterial stiffness • Aging • Endothelial dysfunction • Nitric oxide • Hypertension • Spontaneously hypertensive rats • L-NAME • Pulse wave velocity • Perindopril

Abstract

Introduction: Age and hypertension are two major determinants of arterial stiffness, as well as endothelial dysfunction. The present study was designed to test whether a chronic reduction of endogenous nitric oxide (NO) produces arterial stiffening close to that observed in old spontaneously hypertensive rats (SHR), and also to study the effect of an acute or a chronic decrease in blood pressure (BP) on aortic distensibility. Methods: BP, aortic stiffness, endothelial dysfunction and remodelling were measured in male adult (20-week-old) SHR, in adult SHR treated with a nonspecific NO synthase inhibitor L-NAME (SHR/L-NAME) for 2 weeks, in adult SHR/L-NAME cotreated with perindopril (1 mg/kg/day) and in old SHR (55-week-old). Age-matched WKY were used as a normotensive group. Results: Aortic endothelial dysfunction, remodelling and stiffening appeared in old SHR. Reduction of NO production in adult SHR caused similar alterations. Acute decreases in BP in SHR/L-NAME did not improve isobaric aortic distensibility but a chronic reduction of BP prevented endothelial dysfunction, aortic remodelling and aortic wall stiffening. Conclusion: NO reduction in adult SHR induces aortic alterations similar to those observed during aging, which supports the major role of NO in the development of arterial stiffening. These aortic alterations can be prevented by angiotensin-converting enzyme inhibitor treatment.

Introduction

Hypertension is a major health problem affecting around 1 billion people worldwide and is predicted to increase to 1.5 billion by 2025 [1]. Arterial stiffness has emerged as an important risk factor for cardiovascular diseases and has been entered into the 2007 guidelines for hypertension of the European Society of Hypertension [2] highlighting the importance of taking into account early vascular aging [3] in diagnosis. Epidemiological and clinical studies have shown that increased aortic stiffness, determined by the measurement of aortic pulse wave velocity (PWV), is an independent marker of cardiovascular risk in the general population, an independent predictor of all-cause and cardiovascular mortality in hypertensive patients [4] and a major contributor to the mortality in end-stage renal disease [5]. It has been estab-
lished in patients with end-stage renal disease that despite a similar reduction in mean blood pressure (BP), only survivors had a PWV attenuation [6], pointing out the importance of arterial stiffness for the future development of new drugs [7].

Age and BP are two major determinants of arterial stiffening [8]. Spontaneously hypertensive rats (SHR) have been extensively used in experimental studies because of their clinical relevance for human hypertension [9]. Old SHR naturally develop all the pathophysiological and clinical alterations also noted in patients with essential hypertension [10]. Interestingly, chronic administration of the nonspecific NO synthase inhibitor (L-NAME) at low dose to adult SHR decreases endogenous NO production directly influences arterial stiffness in experimental animals and was reproduced in patients with end-stage renal disease [11]. Spontaneously hypertensive rats (SHR) develop endothelial dysfunction [13, 14], severe hypertensive heart disease [15] (interstitial fibrosis, myocardial infarction, impaired systemic and cardiac haemodynamic) and end-stage renal disease [16] (massive proteinuria, glomerular arteriolar constriction, arteriolar fibrinoid necrosis, glomerular sclerosis) consistent with what is observed in old SHR as well as in untreated hypertensive patients [2].

Clinical studies have indicated that impaired endothelium-dependent vasodilation is associated with an increase in aortic PWV [17–19]. Moreover, inhibition of NO production directly influences arterial stiffness in experimental [20, 21] and clinical studies [22], supporting the fact that endothelium-derived NO regulates arterial stiffness in vivo and may play a major role in the vascular aging process.

Thus, our working hypothesis was that such chronic reduction of endogenous NO production in adult SHR could promote arterial stiffening to a similar level as that observed naturally during aging in hypertensive rats. First, we aimed to compare in SHR the arterial stiffening occurring with aging and with a low dose of L-NAME treatment. Then, in SHR/L-NAME, we investigated the effect of acute and chronic decreases in BP on the vascular wall stiffness.

Methods

Animals and Drug Treatment

This study was conducted in accordance with European Community Guidelines for the use of experimental animals and was approved by the ethical committee on Animal Experiments of the Servier Research Institute. All animals were provided by CERI (Le Gesnay St Isle, France) and were maintained under standard conditions (temperature 22°C and hygrometry 55%). They were given standard chow (RM1®, SDS) and tap water ad libitum. The first part of the study was conducted to compare the effect of age and hypertension on arterial stiffness. Moreover, we evaluated the effect of the decrease in endogenous NO production on arterial stiffness. Thus, three groups of SHR were compared. Twenty-week-old SHR were used – they are hypertensive without arterial damage and are considered to be adult (adult SHR) [23]. The second group was 20-week-old SHR after 2 weeks of treatment with N-nitro-L-arginine methyl ester (L-NAME; Sigma) in drinking water, 50 mg/l, which is the standard dose for this model (SHR/L-NAME) used in previous studies [13, 14, 24]. In the third group, old SHR were used at 50–60 weeks of age – they present marked hypertension and organ damage comparable with human disease [25]. In addition, Wistar Kyoto (WKY) rats were used at 20 and 50–60 weeks of age as a normotensive control group. The second part of the study focused on the effect of an acute and chronic decrease in BP on arterial stiffness in SHR/L-NAME. A group of SHR/L-NAME received a bolus of clonidine (0.3 μg/kg, i.v) to briefly decrease BP; another group of SHR/L-NAME was treated with perindopril (Technologie Servier, Orléans, France) at 1 mg/kg/day in drinking water for 2 weeks together with L-NAME treatment in order to produce a chronic decrease in BP. This dose of perindopril, an angiotensin-converting enzyme (ACE) inhibitor, is known to inhibit the hypertensive response of angiotensin I [26].

Telemetry

Some rats were implanted with a telemetric device (TA11PA-C40, Data Science International, The Netherlands) 3 weeks before the start of the study. To reduce any infection and pain, the rats received one dose of trimethoprim + sulfadoxine (7%, 1 ml/kg s.c.; Borgal®, Virbac, France) and buprenorphine (30 μg/kg s.c.; Buprécare®, Axience, France) just before surgery. Mean arterial BP was sampled and averaged over 10 s every 10 min. Means of the values recorded over a 24-hour period were calculated using a data analysis software (Dataquest ART®, DSI).

PWV and BP Measurements

The rats were anaesthetized with sodium pentobarbital (50 mg/kg i.p.), placed on a warming blanket in order to control their body temperature at 38°C and ventilated after a tracheotomy at a respiratory rate of around 70 breaths per min with an airway pressure of 10 cm H₂O (Hallowell, T.E.M.). A polyethylene catheter (Arterial Leader Cath., VYGON, Ecouen, France) was introduced through the left common carotid artery into the aortic arch to measure central aortic BP. Another polyethylene catheter was inserted into the abdominal aorta until the iliac bifurcation via the left femoral artery to record distal aortic BP. Each catheter was connected to a signal processor (MP100®, Biopac) via two P10EZ pressure transducers (Harvard). After a stabilisation period of 15 min, BP signals were simultaneously recorded online at a sampling rate of 1,000 points/s and then analysed with AcqKnowledge 3.7.3 (Biopac). Central and distal systolic BP, diastolic BP and pulse pressure were measured and recorded on a computer for further analysis. At the end of the recording, the rats were euthanized by a lethal dose of pentobarbital sodium. After dissection, the tips of the two catheters were visually marked and the distance between them was carefully measured. The foot-to-foot method was used to determine the time delay between the central and dis-
nal BP. PWV was calculated as the aortic distance between the tips of the two catheters divided by the time delay and expressed in m s\(^{-1}\). The β-index was calculated according to the following formula: 2.11 × (PWV\(^2\) : central diastolic BP) as an index of arterial stiffness poorly influenced by BP level [20].

**Local Aortic Stiffness Assessment**

Aortic distensibility was assessed on the abdominal aorta. The aortic diameter was measured by the ultrasound detection of the arterial walls using an echotracking device (NIUS-02, Asulab, Switzerland) able to record and to synchronize the aortic diameter with BP. From the pulsatile diameter, defined as the difference between the systolic and diastolic diameter, the relative change in lumen cross-sectional area (LCSA) calculated together with the pulsatile change in blood pressure (ΔP) define the aortic distensibility. Aortic distensibility = (1/LCSA) × (ΔLCSA/ΔP). When ΔP = pulse pressure, the distensibility was defined as operational distensibility. The relationship between BP and the LCSA was fitted with the Langewouters’ model [27]. In order to assess the isobaric distensibility, the aortic distensibility at different arterial pressures over the whole cardiac cycle was calculated and plotted as an arterial-distensibility blood pressure curve. In addition, to avoid the influence of BP level, we measured aortic distensibility after an acute decrease in BP by the sympathetic agent clonidine (0.3 μg/kg i.v., central α\(_2\)-adrenoceptor agonist) in SHR/\(^\text{I}\)-NAME.

**Ex vivo Vascular Reactivity**

The thoracic aorta was removed and immediately placed in cold modified Krebs solution of the following composition (mM): NaCl 118, KCl 2.5, CaCl\(_2\) 2.5, MgSO\(_4\) 1.2, KH\(_2\)PO\(_4\) 1.2, NaHCO\(_3\) 25, EDTA calcium disodium 0.026 and glucose 11.1. Rings (4 mm in length) of the aorta were mounted in organ chambers filled with oxygenated salt solution at 37 °C for isometric tension recording which was digitalized by a computer using IOX software (EMKA Technologies, Paris, France). Then, all segments were submitted to a contraction caused by potassium chloride (KCl: 6.10\(^{-2}\) M). After washing and returning to the baseline, the aortic rings were contracted with the α\(_1\)-adrenoceptor agonist, phenylephrine (10\(^{-7}\) M) and endothelium-dependent and independent relaxations were assessed by cumulative concentration-response curves to acetylcholine (ACh: 10\(^{-6}\)–10\(^{-2}\) M) and sodium nitroprusside (SNP: 10\(^{-10}\)–10\(^{-5}\) M), respectively. Each ring was exposed to only one set of cumulative concentrations of a given agonist. Relaxing responses were expressed as a percentage of the maximal relaxation produced by papaverine (10\(^{-4}\) M) obtained in each ring at the end of the experiment. All drugs were purchased from Sigma-Aldrich (La Verpillère, France) except SNP which was obtained from Prolabo (France).

**Histology**

The thoracic aorta was fixed in 4% neutral-buffered formaldehyde, embedded in paraffin, sectioned at 5 μm thickness and stained with hemalum eosin. Aorta intima-media thickness (IMT) was measured using analysis software (AnalySIS\(^\text{®}\), Olympus). The internal border of the intima and the external border of the media were drawn and the software automatically plotted 150–200 segments perpendicular to these two borders. Two slices were analysed per animal. The average of all these segments was defined as the mean IMT of the aortic section.

**NO Bioavailability**

Nitrosyl hemoglobin (HbNO), an accurate noninvasive marker of endothelial NO production in vivo, was assayed by electron spin resonance [28] in a few rats. Briefly, blood samples were collected on heparin (50 U/ml) and sodium dithionite (20 mg/ml) was added to prevent excessive oxygenation. Red blood cells were collected by centrifugation (10 min, 2,500 g) and HbNO levels were recorded in a finger dewar refrigerated with liquid nitrogen with a Magnettech MS200. System settings were: BO field (3,340 G), BO sweep (300 G), Sweep-time (30 s), Pass (4), Modulation (7,000), MW attenuation (4 db). Spectra were analysed on the Analysis 2.02 software provided with the instrument. Signal amplitude was plotted against a nitrite standard curve to determine sample concentration.
Data and Statistical Analysis
All data followed a Gaussian distribution and were expressed as the mean ± standard error of mean (SEM). However, because of the lognormal distribution of β-index, EC50 and the fibrosis gene, we used a log decimal transformation for their analysis. A two-way ANOVA followed by a Tukey test was performed to analyse the effect of age and strain and their interaction. A Student t test was used to analyse the effect of L-NAME. A one-way ANOVA was used to analyse all comparisons with more than two groups. The statistical analysis was performed with EasyStat using SAS V9.1 software. Significance was p < 0.05 for principal effects.

Table 1. Aortic parameters in adult (20-week-old) WKY, SHR and SHR/L-NAME, and old (55-week-old) WKY and SHR

<table>
<thead>
<tr>
<th></th>
<th>WKY</th>
<th>Old WKY</th>
<th>SHR</th>
<th>SHR/L-NAME</th>
<th>Old SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aortic reactivity</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>ACh relaxation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emax, %</td>
<td>94 ± 3 (5)</td>
<td>76 ± 8.5b (6)</td>
<td>92 ± 4 (8)</td>
<td>47 ± 8c (5)</td>
<td>37 ± 4a,b (5)</td>
</tr>
<tr>
<td>EC50, 10⁻⁸ M</td>
<td>3.1 ± 1.2 (5)</td>
<td>5.4 ± 1.3 (6)</td>
<td>3.5 ± 0.9 (8)</td>
<td>10.8 ± 2.2c (5)</td>
<td>21.1 ± 7.3a,b (5)</td>
</tr>
<tr>
<td>SNP relaxation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emax, %</td>
<td>100 ± 1 (5)</td>
<td>99 ± 0.1 (5)</td>
<td>97 ± 1.5 (8)</td>
<td>98 ± 1 (6)</td>
<td>96 ± 2 (7)</td>
</tr>
<tr>
<td>EC50, 10⁻⁹ M</td>
<td>1.6 ± 0.2 (5)</td>
<td>3.6 ± 2.9 (5)</td>
<td>3.3 ± 1.1 (8)</td>
<td>5.4 ± 1.8 (6)</td>
<td>11.9 ± 1.5a,b (7)</td>
</tr>
<tr>
<td><strong>Aortic thickness/fibrosis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IMT, μm</td>
<td>126 ± 2 (6)</td>
<td>142 ± 7b (6)</td>
<td>135 ± 4 (4)</td>
<td>149 ± 2c (5)</td>
<td>200 ± 2a,b (6)</td>
</tr>
<tr>
<td>TGF-β mRNA</td>
<td>1.4 ± 0.2 (8)</td>
<td>3.0 ± 0.5b (8)</td>
<td>2.0 ± 0.3 (9)</td>
<td>4.9 ± 0.9c (10)</td>
<td>3.5 ± 0.7b (8)</td>
</tr>
<tr>
<td>CTGF mRNA</td>
<td>2.1 ± 0.5 (8)</td>
<td>9.6 ± 2.4b (8)</td>
<td>2.4 ± 0.4 (9)</td>
<td>5.4 ± 0.9c (10)</td>
<td>13.6 ± 3.1b (10)</td>
</tr>
<tr>
<td>PAI-1 mRNA</td>
<td>1.0 ± 0.4 (8)</td>
<td>6.4 ± 1.5b (8)</td>
<td>2.8 ± 0.6a (9)</td>
<td>6.5 ± 1.1c (10)</td>
<td>23.9 ± 6.4a,b (9)</td>
</tr>
<tr>
<td>FN mRNA</td>
<td>0.9 ± 0.2 (8)</td>
<td>3.4 ± 0.9b (7)</td>
<td>1.0 ± 0.2 (9)</td>
<td>6.8 ± 1.2c (10)</td>
<td>13.9 ± 3.9a,b (10)</td>
</tr>
</tbody>
</table>

Data are means ± SEM with the number of animals in parentheses.
Hypertension effect at fixed age levels: a p < 0.05 vs. age-matched WKY, age effect at fixed hypertension levels: b p < 0.05 vs. adult rats, L-NAME effect: c p < 0.05 vs. adult SHR.

TGF-β = Transforming growth factor-β; CTGF = connective tissue growth factor; PAI-1 = plasminogen activator inhibitor-1; FN = fibronectin.

Results

Effect of Hypertension and Age on Aortic Function and Remodelling
We aimed to compare the effect of age and hypertension on aortic function and remodelling. WKY and SHR were compared at 20 and 55 weeks old.

Table 1 summarizes the different aortic parameters. Maximum endothelium-dependent relaxation to ACh was impaired by age in normotensive and hypertensive rats, and the alteration was more pronounced in old hypertensive rats (interaction p = 0.002). No effect of age and hypertension was observed on the maximal endothelium-independent relaxation to SNP. Only old SHR had higher EC50 values to both ACh and SNP indicating an impairment of vascular reactivity with age and hypertension. In addition, we measured some fibrosis genes from the aorta. In both strains TGF-β and connective tissue growth factor were increased with age with no effect of hypertension. In contrast, age in both strains induced PAI-1 and fibronectin genes in the aorta, but PAI-1 and fibronectin were further increased in old SHR compared to old WKY. IMT measured by histology was increased with age in the two strains and exacerbated in old SHR.

Effect of Hypertension and Age on Haemodynamic and Aortic Stiffening
Table 2 shows haemodynamic parameters. Heart rate was lower in all groups of SHR than in WKY. Central and distal BP (systolic and diastolic) was significantly higher in SHR than in WKY and increased with age in both strains. Moreover, central and distal pulse pressure was increased in old SHR. Aortic stiffness assessed by PWV was significantly affected by both age and hypertension (fig. 1a). β-index, a stiffness index poorly influenced by BP level was, as expected, not modified by hypertension as indicated by the comparison between adult SHR and adult WKY (fig. 1b). β-index was significantly modified by age in WKY and more increased in old SHR.

Data and Statistical Analysis
All data followed a Gaussian distribution and were expressed as the mean ± standard error of mean (SEM). However, because of the lognormal distribution of β-index, EC50 and the fibrosis gene, we used a log decimal transformation for their analysis. A two-way ANOVA followed by a Tukey test was performed to analyse the effect of age and strain and their interaction. A Student t test was used to analyse the effect of L-NAME. A one-way ANOVA was used to analyse all comparisons with more than two groups. The statistical analysis was performed with EasyStat using SAS V9.1 software. Significance was p < 0.05 for principal effects.
As expected, administration of L-NAME (6.1 ± 0.2 mg/kg/day) in adult SHR induced a reduction of endogenous NO production, assessed by a decrease in HbNO (measured by electron spin resonance) in SHR/L-NAME compared with untreated SHR [HbNO: 163 ± 30 (n = 7) vs. 645 nM ± 104 (n = 6), respectively, p < 0.05]. L-NAME administration caused a marked endothelial dysfunction in SHR characterized by both a decrease in maximum relaxation and a higher EC_{50} value to ACh (table 1). In addition, the fibrosis gene expression measured in aorta as well as the aortic IMT was increased in adult SHR treated with L-NAME compared with untreated adult SHR (table 1).

**Effect of L-NAME in SHR on Haemodynamic and Aortic Stiffening**

Central and distal blood pressure (systolic, diastolic) was significantly increased by L-NAME (table 2). Central but not distal pulse pressure was significantly increased in SHR/L-NAME compared with SHR (table 2). Both

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**Table 2. Haemodynamic parameters in adult (20-week-old) WKY, SHR and SHR/L-NAME, and old (55-week-old) WKY and SHR**

<table>
<thead>
<tr>
<th></th>
<th>WKY</th>
<th>Old WKY</th>
<th>SHR</th>
<th>SHR/L-NAME</th>
<th>Old SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, bpm</td>
<td>427 ± 13 (10)</td>
<td>427 ± 12 (11)</td>
<td>362 ± 12(^{a}) (11)</td>
<td>363 ± 8 (10)</td>
<td>349 ± 8(^{a}) (10)</td>
</tr>
<tr>
<td>Central pressure, mm Hg</td>
<td>154 ± 4 (10)</td>
<td>181 ± 8(^{b}) (11)</td>
<td>201 ± 7(^{a}) (11)</td>
<td>238 ± 10(^{c}) (10)</td>
<td>252 ± 13(^{a,b}) (10)</td>
</tr>
<tr>
<td>Systolic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Diastolic</td>
<td>124 ± 3 (10)</td>
<td>144 ± 4(^{b}) (11)</td>
<td>160 ± 5(^{a}) (11)</td>
<td>183 ± 5(^{c}) (10)</td>
<td>181 ± 7(^{a,b}) (10)</td>
</tr>
<tr>
<td>Pulse</td>
<td>30 ± 2 (10)</td>
<td>37 ± 5 (11)</td>
<td>39 ± 3 (11)</td>
<td>54 ± 6(^{c}) (10)</td>
<td>71 ± 7(^{a,b}) (10)</td>
</tr>
<tr>
<td>Distal pressure, mm Hg</td>
<td>165 ± 3 (10)</td>
<td>191 ± 7(^{b}) (11)</td>
<td>206 ± 6(^{a}) (11)</td>
<td>237 ± 10(^{c}) (10)</td>
<td>258 ± 11(^{a,b}) (10)</td>
</tr>
<tr>
<td>Systolic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic</td>
<td>120 ± 2 (10)</td>
<td>138 ± 4(^{b}) (11)</td>
<td>157 ± 4(^{a}) (11)</td>
<td>178 ± 5(^{c}) (10)</td>
<td>179 ± 7(^{a,b}) (10)</td>
</tr>
<tr>
<td>Pulse</td>
<td>46 ± 3 (10)</td>
<td>53 ± 4 (11)</td>
<td>51 ± 3 (11)</td>
<td>59 ± 6 (10)</td>
<td>80 ± 5(^{a,b}) (10)</td>
</tr>
</tbody>
</table>

Data are means ± SEM with the number of animals in parentheses. Hypertension effect at fixed age levels: \(^{a}\) p < 0.05 vs. age-matched WKY, age effect at fixed hypertension levels: \(^{b}\) p < 0.05 vs. adult rats, L-NAME effect: \(^{c}\) p < 0.05 vs. adult SHR.

**Fig. 1.** PWV (a) and β-index (b) in adult WKY (20-week-old, n = 8), SHR (n = 8), SHR treated with L-NAME (SHR/L-NAME, n = 9), old WKY (55-week-old, n = 11) and SHR (n = 10). Data are mean ± SEM. Hypertension effect at fixed age levels \(^{a}\) p < 0.05 versus age-matched WKY, age effect at fixed hypertension levels: \(^{b}\) p < 0.05 versus adult rats, L-NAME effect: \(^{c}\) p < 0.05 versus adult SHR.
PWV (fig. 1a) and β-index (fig. 1b) were increased in adult SHR treated with L-NAME compared to adult untreated SHR.

Effect of Acute and Chronic BP Reduction in SHR/L-NAME

In this second part of the present work, we studied the possible BP dependency of the local arterial stiffness using the echotracking method. Therefore, from the arterioal-distensibility BP curve shown in figure 2a, we could determine the isobaric distensibility at 170 mm Hg (fig. 2b). Under basal conditions, isobaric distensibility was strongly reduced in SHR/L-NAME compared to SHR. Acute clonidine administration in SHR/L-NAME reduced the systolic blood pressure to a similar level to that observed in SHR (193 ± 6 vs. 206 ± 6 mm Hg, respectively) but did not improve isobaric distensibility. All these results allowed us to confirm that the arterial stiffening observed in SHR treated with L-NAME was due to an increase in vascular wall stiffening.

To further test this hypothesis, we evaluated arterial stiffening in SHR/L-NAME under an antihypertensive and vasoprotective treatment. Chronic perindopril administration completely prevented the rise in BP, measured by telemetry, due to L-NAME in SHR (fig. 3a), maintaining BP at a similar level as in untreated SHR. Perindopril administration in SHR/L-NAME helped to prevent the body weight loss (365 ± 5 g vs. 304 ± 12 g) and the mortality rate (0 vs. 14%; table 3). As indicated in figure 3b, perindopril prevented the increase in PWV observed in untreated SHR/L-NAME. Figure 3d illustrates that chronic perindopril treatment restored the isobaric aortic distensibility in SHR/L-NAME indicating a positive impact on the arterial stiffening. In addition, perindopril treatment prevented endothelial dysfunction, aortic IMT and the increase in fibrosis gene expression compared to untreated SHR/L-NAME (table 3), confirming the beneficial effect of perindopril on vascular wall remodelling.

Discussion

The main findings of the present study are that: (1) Chronic reduction of NO production in adult SHR by a low dose of the NO synthase inhibitor L-NAME causes aortic stiffening comparable to that observed in old SHR, associated with endothelial dysfunction, aortic remodelling and fibrosis, and (2) Chronic but not acute decreases in BP have protective effects on aortic stiffening, likely via the associated reduction of endothelial dysfunction and aortic remodelling. Together, the data suggest that
aortic stiffening in the SHR/L-NAME is at least in part BP independent and due to vascular wall remodelling and endothelial dysfunction.

Age and BP are two major determinants of arterial stiffening [8]. In the last decade endothelial dysfunction has appeared as a possible factor promoting arterial stiffness. Endothelium-dependent vasodilatation is impaired with aging in normotensive subjects and further reduced in essential hypertensive patients [29]. Acute inhibition of NO production directly influences arterial stiffness parameters as observed in experimental [20, 21] and clinical studies [22], supporting the hypothesis that endothelium-derived NO regulates arterial stiffness in vivo and may play a major role in the vascular aging process.

In the first part of the study, we characterized the aortic alterations occurring with age and hypertension. Old SHR is a useful experimental model of hypertension, known to present marked hypertension and organ damage comparable with human disease [25]. We observed that endothelial function is impaired by aging in WKY
and is more severe in old SHR as indicated by a decrease in relaxation responses to acetylcholine. Moreover, we showed that an aortic remodelling and fibrosis, assessed by aortic wall thickness and fibrosis gene expression, occurs with age in WKY and is more pronounced in old SHR. In accordance with Marque et al. [23], the intrinsic aortic stiffness is not increased in adult SHR compared with age-matched WKY but is largely influenced by age and exacerbated in old SHR as demonstrated by an increase in PWV and $\beta$-index (a BP independent arterial stiffness parameter). We also observed an increase in central BP as a consequence of the increased arterial stiffness in old SHR. Previous studies have suggested that aortic IMT and wall composition in old SHR cannot fully explain the aortic stiffening which might be the result of an adaptation of the blood vessel wall to increased BP [23, 30]. In addition, our results in old SHR are also in line with the hypothesis that endothelial dysfunction may play a major role in the early vascular aging in SHR and a lack in NO may participate in this phenomenon. Indeed, a lesser NO availability may lead to a loss of the antihypertrophic and antiproliferative influence of NO and thus result in structural modifications within the arterial wall characterized by a high content of stiffer elements, such as collagen and calcification reinforcing the intrinsic arterial wall stiffness [31–33].

In parallel, we performed similar experiments in SHR treated with a low dose of an NO synthase inhibitor which is approximately 1/20 of the dose used to increase BP in normotensive rat. The dose used here is the usual dose administrated in this model in previous studies [13, 14, 24] and allows the significant reduction of the 24-hour urinary NO excretion [11] as well as in the present study the circulatory NO. It has been shown that such L-NAME administration in adult SHR induces severe hypertensive heart disease [15] and end-stage renal disease [16] consistent with observations made in untreated hypertensive patients [24]. This experimental model appears valuable in improving our knowledge of the role of NO in arterial stiffness regulation. Administration of L-NAME in adult SHR caused an endothelial dysfunction, an increase in BP, in aortic IMT and in fibrosis factors. These alterations were associated with a pronounced arterial stiffening measured by increases in PWV and in $\beta$-index. Interestingly, all these changes in SHR/L-NAME resembled those noted in old SHR. Thus, our results help to demonstrate that chronic reduction of endogenous NO production in adult SHR mimics the effect of aging and, for the first time, that SHR/L-NAME may be a good experimental model of early vascular aging.

In the second part of the study, to better characterize aortic stiffening in SHR/L-NAME, we measured arterial distensibility using an echotracking technique which simultaneously analyses BP and aortic diameter systolic-diastolic changes and allows the determination an isobaric distensibility. We did not observe a difference in distensibility between SHR and WKY in isobaric conditions. However, in SHR/L-NAME we first noticed a lower

**Table 3. Effect of perindopril in SHR/L-NAME**

<table>
<thead>
<tr>
<th></th>
<th>SHR</th>
<th>SHR/L-NAME</th>
<th>SHR/L-NAME + perindopril</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight, g</strong></td>
<td>368 ± 5 (6)</td>
<td>304 ± 12a (5)</td>
<td>365 ± 5b (8)</td>
</tr>
<tr>
<td><strong>Aortic reactivity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACh relaxation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emax, %</td>
<td>82 ± 4 (5)</td>
<td>54 ± 6a (5)</td>
<td>81 ± 8b (6)</td>
</tr>
<tr>
<td>EC_{50}, 10^{-8} M</td>
<td>2.5 ± 0.2 (5)</td>
<td>9.4 ± 3.1a (5)</td>
<td>2.9 ± 0.8b (6)</td>
</tr>
<tr>
<td><strong>Aortic thickness/fibrosis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IMT, μm</td>
<td>144 ± 2 (6)</td>
<td>177 ± 5a (6)</td>
<td>160 ± 4b (6)</td>
</tr>
<tr>
<td>CTGF mRNA</td>
<td>-</td>
<td>1.26 ± 0.18 (15)</td>
<td>0.80 ± 0.14b (17)</td>
</tr>
<tr>
<td>PAI-1 mRNA</td>
<td>-</td>
<td>1.13 ± 0.17 (15)</td>
<td>0.74 ± 0.15b (17)</td>
</tr>
<tr>
<td>FN mRNA</td>
<td>-</td>
<td>1.04 ± 0.17 (15)</td>
<td>0.33 ± 0.07b (17)</td>
</tr>
<tr>
<td>Mortality, %</td>
<td>-</td>
<td>14 (3/22)</td>
<td>0 (0/29)</td>
</tr>
</tbody>
</table>

Data are means $\pm$ SEM with the number of animals in parentheses.
One-way ANOVA, $^a$ p < 0.05 vs. SHR, $^b$ p < 0.05 vs. untreated SHR/L-NAME.

CTGF = Connective tissue growth factor; PAI-1 = plasminogen activator inhibitor-1; FN = fibronectin.
isobaric distensibility than in SHR under basal conditions which confirms the aortic wall stiffening. To further complete our observations, we performed an acute decrease in BP in order to better characterize the arterial wall stiffness independently of the BP component. In accordance with a previous study by our research group [34], an acute decrease in BP by a bolus administration of clonidine did not improve aortic isobaric distensibility in SHR/L-NAME. In contrast a chronic BP reduction by a 2-week treatment with perindopril improves it, demonstrating that transmural pressure per se is not the major factor but that the long-term elevation of BP associated with vascular wall alteration is responsible for the aortic stiffening in SHR/L-NAME. Perindopril is known to have a beneficial impact on endothelial dysfunction [35, 36] and has been tested previously on arterial stiffness in a hypertensive population [37]. In a preliminary study, at 1 mg/kg/day, perindopril produced a strong inhibition of ACE activity. This dose of perindopril given to SHR/L-NAME completely prevented the BP rise caused by L-NAME and reduced mortality. This beneficial effect on the vascular wall stiffening could be explained by the complete recovery of endothelial dysfunction, and/or the reduction of arterial remodelling and thickening also observed in the rats treated with perindopril. Taken together, our data indicate that a substantial part of the arterial stiffening observed in SHR/L-NAME results from the overall aortic wall alterations described above.

In summary, the induction of endothelial dysfunction in adult SHR via a reduction of NO production leads to remodelling and stiffening of the aorta similar to that observed in old SHR. Moreover, prevention of endothelial dysfunction and hypertension by the ACE inhibitor is associated with prevention of arterial remodelling and stiffening.

In conclusion, our study demonstrates that endothelial dysfunction, due to chronic reduction of NO, plays a major role in the early vascular aging in hypertensive rats and shows that SHR/L-NAME is a useful animal model for study of the mechanisms involved in arterial stiffness.

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References


